REMARKS

Claims 1 and 2 have been amended to recite "wherein said enzyme has (1) the amino acid sequence of SEQ ID NO: 2 or (2) an amino acid sequence encoded by the DNA sequence of SEQ ID NO: 1 or (3) an amino acid sequence encoded by a DNA sequence that hybridizes to the complement of DNA sequence of SEQ ID NO: 1 under highly stringent hybridization conditions of 6X SSC, 0.5% SDS, 100 µg/ml denatured salmon sperm DNA, 50% formamide, and incubating overnight at 42°C with gentle rocking and highly stringent wash conditions of washing in 2X SSC, 0.5%SDS at room temperature for 15 minutes, followed by another wash in 0.1X SSC, 0.5% SDS at room temperature for 15 minutes and having the activity to produce L-ascorbic acid." Support for this amendment may be found in the specification at, for example, page 7, lines 16-21.

It is submitted that no new matter has been introduced by the foregoing amendments. Approval and entry of the amendments is respectfully solicited.

Indefiniteness Rejection

Claims 1, 2, 6, 7, and 13 were rejected under 35 USC §112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." (Paper No. 20080124 at 4). In making the rejection, the Examiner asserted that the metes and bounds of the claims are not clear because they recite "...under stringent hybridization and wash conditions...". (*Id.*) The Examiner further asserted that "[w]hile page 4 of the specification describes some conditions which are intended to be stringent, there is nothing to suggest that other conditions would not also be included within the scope of

this term and in the art what is considered stringent varies widely depending on the individual situation as well as the person making the determination." (*Id.* at 4-5) The Examiner required clarification and correction.

In accordance with the Examiner's instructions, claims 1 and 2 have been amended to recite specific highly stringent conditions. As is well settled, all that is required to comply with 35 USC §112, second paragraph, is that the metes and bounds of what is claimed be determinable with a reasonable degree of precision and particularity. *Ex parte Wu*, 10 USPQ2d 2031, 2033 (BPAI 1989). The applicants respectfully submit that the metes and bounds of the amended claims 1 and 2 are determinable because hybridization and wash conditions are specified in these claims. Therefore, the indefiniteness rejection is rendered moot and should be withdrawn.

Written Descirption/New Matter Rejection

Claims 1, 2, 6, 7, 8, 13, and 16 were rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. (Paper No. 20080124 at 2).

In making the rejection, the Examiner asserted that "[c]laims 1, 2, 8 are rejected because the phrase 'directly' is new matter." (*Id.*) The Examiner also asserted that "[t]he scope of the process for the production of L-ascorbic acid comprising: (a) contacting an enzyme with a substrate which is selected from the group consisting of L-gulose, L-galactose, L-iodose and L-talose; (b) converting the substrate 'directly' into L-ascorbic acid .... as claimed was not contemplated in the specification as originally filed." (*Id.*, emphasis original) The Examiner further asserted that "[a]pplicants' claim to

have support for this amendment in the specification at for example, page 1, lines 1-2; page 2, lines 8-12; page 2, line 25 to page 3, line 9 and lines 27-29; in examples 1-4 and Tables 1-4 and in original claims 1 and 5 without addressing the issues raised by the examiner in the Office action dated 05/11/2007." (*Id.* at 2-3). Additionally, the Examiner claims to be "unable to find either explicit or implicit meaning wherein the claims or the specification construes/contemplates converting the substrates directly into L-ascorbic acid by catalytic activity of a polypeptide having the amino acid sequence of SEQ ID NO: 2." (*Id.* at 3).

Furthermore, the Examiner asserted that in the claims, "comprising [] is interpreted as 'open language' and therefore the process for production of L-ascorbic acid can comprise other elements in the reaction." (*Id.*, emphasis original.) The Examiner also asserted that

> [t]he said process of production of L-ascorbic acid was carried out under specific cellular context *in vivo,* i.e., production of L-ascorbic acid in a process comprising: contacting an enzyme having the amino acid sequence of SEQ ID NO: 2 encoded by a polynucleotide of SEQ ID NO: 1, said polypeptide expressed in a specific strain of *E. coli* JM 109 having the activity to produce L-ascorbic acid from substrates L-gulono-1,4-lactone/L-gulonic acid from L-gulose and from L-galactono-1,4-lactone/L-galctonic acid or conversion of substrate L-galactose to L-galactono-1,4-lactone/L-galactoriic acid and L-ascorbic acid under suitable culture conditions (as in Examples: 1-4 and Tables 1-4, pages 8-10; and culture conditions: lines 15-28, page 6 of specification) and therefore said bacteria may provide other necessary enzymes either for the production of intermediate products of L-ascorbic with claimed substrates or for the final conversion of the intermediate products to L-ascorbic acid. (*Id.* at 3-4)

The Examiner concluded, "[t]herefore said process of production of L-ascorbic acid does not involve converting substrates directly into L-ascorbic acid." (*Id.* at 4)

The Examiner also asserted that "[the] claimed recitation of a use of Enzyme B ... (in page 1, lines 1-2 of specification), without setting forth any steps involved in the process, results in an improper definition of a process" and that "[t]he recitation of [the] phrase "use" without any active, positive steps delimiting how this use is actually practiced, renders the term indefinite and failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention" (*Id.*, emphasis original.)

The rejection is respectfully traversed.

Initially, we note that the Examiner's assertion regarding the "improper definition of a process" refers to **not to any claim** of the application, but to **the first page of the specification**. 35 U.S.C. § 112 states, "[t]he specification shall conclude with one or more **claims** particularly pointing out and distinctly **claiming** the subject matter which the applicant regards as his invention." This requirement is clearly directed towards **claims**. There is no requirement that other portions of the specification should distinctly claim the subject matter. Therefore, it is respectfully submitted that the rejection related to the "improper definition of a process" is inapplicable and should be withdrawn.

The applicants also respectfully submit that the phrase "directly" is **not** new matter. Support for the amendments may be found in the specification and in original claims.

A. Original Claims

We note that the original claims 1 recited, "[a] process for the production of L-ascorbic acid with an enzyme having the amino acid sequence of SEQ ID NO: 2 or

an amino acid sequence that is 90% identical thereto, **with the activity to produce L-ascorbic acid**, whereby L-ascorbic acid is produced from a substrate which is selected from the group consisting of L-gulose, L-galactose, L-idose, and L-talose." (emphasis added). Similarly, original claim 2 also recited "[a] process for the production of L-ascorbic acid with an enzyme having the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is 90% identical thereto, **with the activity to produce L-ascorbic acid,** whereby L-ascorbic acid is produced from a substrate which is selected from the group consisting of L-gulono-1, 4-lactone, L-gulonic acid, L-galactono-1, 4-lactone, L-galactonic acid, L-idono-1, 4-lactone, L-idonic acid, L- talon-1, 4-lactone, and L-talonic acid." (emphasis added). Original claim 5 depends from claims 1-4, further recited, *inter alia*, that the product was isolated from the reaction mixture. Thus, the specification clearly supports the current claims, *e.g.* claims 1 and 2, which recite converting the substrate directly into L-ascorbic acid by the catalytic activity of enzyme B or an enzyme that is at least 90% identical to enzyme B. The original claim is clear that the process of the present application does not require any additional chemical step or isolation of an intermediate in order to arrive at the final product L-ascorbic acid.

B. Specification

Likewise, the specification provides support for the addition of the phrase, "directly." Page 3 lines 8-12 of the specification discloses "a process for the production of L-ascorbic acid with an enzyme having the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is 90% identical thereto, **with the activity to produce L-ascorbic acid,** whereby L-ascorbic acid is produced from a substrate..." Page 2, line 25 to page 3, line 9 of the specification discloses

> ...a process for
> (a) producing
> (i) L-ascorbic acid from L-gulose or L-galactose by an enzyme which comprises contacting L-gulose or L-galactose with an enzyme having an amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is 90% identical thereto, or a portion thereof, **with the activity to produce L-ascorbic acid from L-gulose and L-galactose** or a functional equivalent thereof, in a reaction mixture,
> ...or
> (iii) L-ascorbic acid from L-gulono-1,4-lactone or from L-galactono-1,4-lactone by an enzyme which comprises contacting L-gulono-1,4-lactone or from L-galactono-1,4-lactone with the enzyme having an amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is 90% identical thereto, or a portion thereof, **with the activity to produce L-ascorbic acid from L-gulono-1,4-lactone and from L-galactono-1,4-lactone**, or a functional equivalent thereof, in a reaction mixture...

Similar language may also be found on page 3, lines 10 to 16, and page 4, lines 9 to 21. Furthermore, the specification is clear that enzyme B, which has the physico-chemical properties listed on page 1 lines 24 to 29, has the sequence of SEQ ID NO:2. Therefore, the specification is also explicit with respect to converting the substrates directly into L-ascorbic acid by the catalytic activity of enzyme B and supports the addition of the phrase "directly" to claims 1, 2 and 8.

Furthermore, some of the examples are supportive of this direct, one-step conversion to L-ascorbic by enzyme B. In Example 1 (pages 8-9) of the instant application, *E. coli* JM109 carrying pTrcMalE-EnzB (which contains the sequence of enzyme B in a vector with a lac promoter) was cultivated with or without IPTG in the presence of L-gulose. The results of the experiment are shown in Table 1 on page 9, which is reproduced below for the convenience of the Examiner.

TABLE 1

Strain	IPTG	HPLC	
		L-GuL + L-GuA (mM)	Vitamin C (mg/L)
JM109/pTrcMalE-EnzB	+	8.4	42.5
	-	4.6	10.5
JM109	+	Nd	nd
	-	Nd	nd

L-GuL: L-gulono-1,4-lactone; L-GuA: L-gulonic acid; nd: not detected

The Examiner asserts that JM109 "may provide other necessary enzymes either for the production of intermediate products of L-ascorbic [acid] with claimed substrates or for the final conversion of the intermediate products to L-ascorbic acid." (Paper No. 20080124 at 4). Table 1 clearly refutes the possibility that the bacterial enzymes produce intermediate products of L-ascorbic acid, because L-gulono-1,4-lactone and L-gulonic acid are not detected when JM109 alone is used.

Example 1 also counters the Examiner's assertion that the bacteria may provide enzymes for the final conversion of the intermediate products to L-ascorbic acid (Vitamin C) and that enzyme B is not responsible for the conversion of the substrate to L-ascorbic acid. If Examiner's assertion were true, one would expect that intermediates increase at the same rate as or at a lower rate (if bacterial enzymes' conversion of intermediates is rate-limiting) than the accumulation of L-ascorbic acid. But Example 1 clearly shows that upon induction with IPTG, the amount of L-gulono-1,4-lactone and L-gulonic acid increased less than 2 fold, whereas Vitamin C production increased more than 4 fold. This experiment demonstrates that *E. coli* enzymes alone can not explain

such a disproportionate increase in production of vitamin C. Such an increase is directly related to the expression of enzyme B. Therefore, enzyme B is responsible for the conversion of the substrate into Vitamin C.

Therefore, the specification also supports the addition of the phrase "directly" in claims 1, 2 and 8.

C. International Preliminary Examination Report

Furthermore, the International Preliminary Examination Report of the instant application clearly recognizes such a direct, one step process. The Report repeatedly stated that the present application relates the use of enzyme B having the amino acid sequence of SEQ ID NO:2 for the production of L-ascorbic acid from suitable precursors in **one step**. See *e.g.*, Form PCT/Separate Sheet/409, item V, section 2.1.

As set forth above, the specification and original claims support the addition of the phrase "directly." This interpretation is also supported by the International Preliminary Examination Report of the instant application. Therefore, it is respectfully submitted that the phrase "directly" is not new matter. Accordingly, the written description rejection should be withdrawn.

Enablement Rejection

Claims 1-2, 6-8, 13, and 16 were rejected under 35 USC §112, first paragraph, on the asserted grounds that the specification is not enabling. (Paper No. 20080124 at 4).

In making the rejection, the Examiner acknowledged that

> the specification ... [is] enabling for the production of L-ascorbic acid comprising: contacting an enzyme having the amino acid sequence of SEQ ID NO: 2 encoded by a polynucleotide of SEQ ID NO: 1, said polypeptide expressed in a specific strain of *E. coli* JM 109 having the activity to produce L-ascorbic acid from substrates L-gulono-1,4-lactone/L-gulonic acid from L-gulose and from L-galactono-1,4-lactone/L-galctonic [*sic*] acid or conversion of substrate L-galctose [sic] to L-galactono-1,4-lactone/L-galactonic acid and L-ascorbic acid under suitable culture conditions (as in Examples: 1-4, pages 8-10; and culture conditions: lines 15-28, page 6 of specification). (*Id.*)

The Examiner further acknowledged that "methods to produce variants of a known sequence, such as site-specific mutagenesis, random mutagenesis, etc., are well known to the skilled artisan." (*Id.* at 6.)

However, the Examiner asserted that

> the specification does not reasonably provide enablement for a process for the production of L-ascorbic acid comprising: contacting an enzyme with a substrate selected from the group consisting of L-gulose, L-galactose, L-idose, L-talose, L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone, L-galactonic acid, L-idono-1,4-lactone, L-idonic acid, Ltalono- 1,4-lactone and L-talonic acid and converting the substrate directly into L-ascorbic acid by catalytic activity of the enzyme under suitable culture conditions, wherein said enzyme has an amino acid sequence 90% identical to SEQ ID NO: 2 from any source including variants, mutants and recombinants and encoded by the polynucleotide of SEQ ID NO: 1 or an amino acid. sequence encoded by a polynucleotide that hybridizes to SEQ ID NO: 1 under undefined stringent hybridization and wash conditions and further said polypeptide expressed in any cellular context is able to produce L-ascorbic acid in a process for the production of L-ascorbic acid under specific defined process conditions such as pH, temperature and time in which said substrates are allowed to react with said enzyme. (*Id.*, emphasis original.)

The Examiner further asserted that

> producing variants capable of being used in a process for the production of L-ascorbic acid comprising: contacting an enzyme with a substrate selected from the group consisting of L-gulose, L-galactose, L-idose, L-talose, L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone, L-galactonic acid, L-idono-1,4-lactone, L-idonic acid, L-talono-1,4-lactone and L-talonic acid, wherein said enzyme has an amino acid sequence

> 90% identical to SEQ ID NO: 2 from any source including variants, mutants and recombinants and encoded by the polynucleotide of SEQ ID NO: 1 or an amino acid encoded by a polynucleotide that hybridizes to SEQ ID NO: 1 under stringent hybridization conditions and said polypeptide under any conditions i.e., said polypeptide expressed in any cellular context is able to produce L-ascorbic acid in a process for the production of L-ascorbic acid under specific defined process conditions such as pH, temperature and time in which any substrate i.e., selected from the group consisting of L-gulose, L-galactose, L-idose, L-talose, L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone, L-galactonic acid, L-idono-1,4-lactone, L-idonic acid, L-talono-1,4-lactone and L-talonic acid are allowed to react with said enzyme, requires that one of ordinary skill in the art know or be provided with guidance for the selection of which, of the infinite number of variants, have the activity. (*Id.* at 6-7)

Additionally, the Examiner asserted, "[w]ithout such guidance, one of ordinary skill would be reduced to the necessity of producing and testing all of the virtually infinite possibilities." (*Id.* at 7) The Examiner then concluded that "[f]or the rejected claims, this would clearly constitute **undue** experimentation." (*Id.*, emphasis original.) The Examiner also asserted that according to the estimation method disclosed in Guo et al., (PNAS, 2004, Vol. 101 (25): 9205-9210) ("Guo"), "only $(0.66)^{58}$ X 100% or 3.44×10^{-9}% of random mutants having 90% identity would be active." (*Id.*, emphasis original.) The Examiner further asserted that "[c]urrent techniques in the art would allow for finding a reasonable number of active mutants within hundred thousand inactive mutants, [b]ut finding a few mutants within several billions or more, as in the claims to 90% identity, would not be possible." (*Id.* at 7-8.) Moreover, the Examiner asserted, "[a]pplying this estimate to the instant protein, a functional equivalent thereof with 90% sequence identity, as recited in [c]laims 1-2, 6-8, 13 and 16, an extremely low number of active mutants will be present among an enormously large number of inactive mutants and as

such screening for these active mutants would be burdensome and undue experimentation when there is no guidance provided in the specification." (*Id.* at 9.)

Initially, we note that with a view towards furthering prosecution, claims 1 and 2 have been amended to delete recitations of an amino acid sequence with 90% sequence identity to SEQ ID NO: 2 and with the activity to produce L-ascorbic acid. Thus, the Examiner's rejection with respect to such an amino acid sequence are rendered moot.

Additionally, we note that claims 1-2 have been amended to recite specific hybridization conditions. In view of this amendment, the Examiner's concern regarding the alleged "undefined stringent hybridization and wash conditions" has been rendered moot.

Indeed, the use of an enzyme having the amino acid sequence encoded by a DNA sequence that hybridizes to the complement of DNA sequence of SEQ ID NO: 1 under hybridization and wash conditions recited in the in amended claims 1 and 2 is enabled. As acknowledged by the Examiner, "methods to produce variants of a known sequence, such as site-specific mutagenesis, random mutagenesis, etc., are well known to the skilled artisan." (Paper No. 20080124 at 6.) Additionally, the specification discloses which amino acid substitutions may be made: "[a]mino acid exchanges in proteins and peptides which do not generally alter the activity of such molecules are known in the state of the art. The most commonly occurring exchanges are: Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, Asp/Gly as well as these in reverse." (Page 4, line 5-8). The specification also contains four examples

(Examples 1-4) that include assays for identifying enzymes that meet the recited elements of the claims.

In sum, the specification and knowledge in the art provide ample guidance to allow one of skill in the art to practice the currently claimed invention without undue experimentation. Accordingly, the rejection should be withdrawn.

Rejections under 35 U.S.C. § 102(b):

Claims 2, 8, 13, and 16 were rejected under 35 USC § 102(b) as anticipated by Sugisawa *et al.*, "Isolation and Characterization of New Vitamin C Producing Enzyme (L-gulono-g-lactone dehydrogenase) of Bacterial Origin," Biosci. Biotech. Biochem., 1995, Vol. 59(2): 190-196 ("Sugisawa"). (Paper No. 20080124 at 10).

For the reasons set forth below, the rejection is traversed.

In making the rejection, the Examiner asserted that Sugisawa "disclose[s] the purification, kinetic profiles and physico-chemical characterization of a polypeptide derived from *G.oxydans* DSM 4025 that produced L-ascorbic acid from L-gulono-γ-lactone said enzyme consisted [*sic*] of 3 subunits of molecular weight of about 61,000 +/- 1000, 32,500 +/- 1000 and 16,500 +/- 500 with identical physico-chemical properties and substrate specificity, optimal pH range, pH stability, thermal stability and effect of metal and inhibitors on the activity of said enzyme (Abstract section)." (*Id.* at 11.) The Examiner further asserted that "Tables: I, II, IV, V, VI and VII disclose production of L-ascorbic acid, substrate specificity, effects of temperature, pH and various metals on the activity of said enzyme." (*Id.*) The Examiner further asserted that "[c]laims 2, 13 and 16

are included in the rejection although said claims recite specific SEQ ID NO: 2 and the activity of said polypeptides under specific pH and temperature, because examiner interprets these properties to be inherent in the isolated polypeptide." (*Id.*) The Examiner then concluded that Sugisawa "anticipates" claims 2, 8, 13, and 16. (*Id.*) The Examiner also asserted that Sugisawa discloses "a polypeptide derived from *G. oxydans* DSM 4025 that produced L-ascorbic acid from L-gulono-γ-lactone." (*Id.* at 12.)

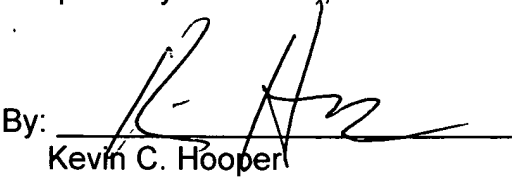
As stated in the response dated November 12, 2007, ***Sugisawa does not even disclose the claimed enzyme***. As admitted by the Examiner, the enzyme disclosed in Sugisawa is approximately 110 kDa consisting of 3 subunits approximately 61,000 +/- 1000, 32,500 +/- 1000, and 16,500 +/- 500 Da, respectively. (*Id.*) In contrast, the claimed enzyme, Enzyme B, consists of only 1 subunit of about 60 kDa. A further difference between the enzyme of the instant application and the enzyme disclosed in Sugisawa is the activity of the enzyme on the substrate L-galactono-1,4-lactone. Table VIII of Sugisawa discloses that the isolated enzyme has no activity on the substrate L-galactono-1,4-lactone (L-galactono-γ-lactone), whereas the claimed enzyme clearly displays activity on this substrate. (*See* Sugisawa at page 195, Table VIII vis-à-vis Example 4, Table 4 of the Specification).

Anticipation requires "identity of invention." *Glaverbel Societe Anonyme,* 33 USPQ2d at 1498. The enzyme disclosed by Sugisawa is clearly not identical to the claimed enzyme. Accordingly, the rejection is insufficient as a matter of law and fact to support a conclusion of anticipation, and for this reason, the rejection should be withdrawn.

Accordingly, for the reasons set forth above, reconsideration, withdrawal of the rejection, and allowance of the claims are respectfully requested. If the Examiner has any questions regarding this paper, please contact the undersigned.

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on August 1, 2008.

Respectfully submitted,

By: 

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